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Commentary

Therapeutic epilepsy research: From pharmacological rationale to focal adenosine augmentation

Detlev Boison *, Kerry-Ann Stewart

Robert Stone Dow Neurobiology Laboratories, Legacy Research, 1225 NE 2nd Ave, Portland, OR 97232, USA

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ABSTRACT

Epilepsy is a common seizure disorder affecting approximately 70 million people worldwide. Current pharmacotherapy is neuron-centered, frequently accompanied by intolerable side effects, and fails to be effective in about one third of patients. Therefore, new therapeutic concepts are needed. Recent research suggests an astrocytic basis of epilepsy, presenting the possibility of novel therapeutic targets. In particular, dysfunction of the astrocyte-controlled, endogenous, adenosine-based seizure control system of the brain is implicated in seizure generation. Thus, astrogliosis – a pathological hallmark of the epileptic brain – is associated with upregulation of the adenosine-removing enzyme adenosine kinase (ADK), resulting in focal adenosine deficiency. Both astrogliotic upregulation of ADK in epilepsy and transgenic overexpression of ADK are associated with seizures, and inhibition of ADK prevents seizures in a mouse model of pharmacoresistant epilepsy. These findings link adenosine deficiency with seizures and predict that adenosine augmentation therapies (AATs) will likely be effective in preventing seizures. Given the wide-spread systemic and central side effects of systemically administered AATs, focal AATs (i.e., limited to the astrogliotic lesion) are a necessity. This Commentary will discuss the pharmacological rationale for the development of focal AATs. Additionally, several AAT strategies will be discussed: (1) adenosine released from silk-based brain implants; (2) adenosine released from locally implanted encapsulated cells; (3) adenosine released from stem cell-derived brain implants; and (4) adenosine augmenting gene therapies. Finally, new developments and therapeutic challenges in using focal AATs for epilepsy therapy will critically be evaluated.

1. Introduction

Epilepsy is a heterogeneous syndrome characterized by excessive electrical discharges in neuronal clusters that result in spontaneous and recurrent seizures. Although it is one of the most prevalent neurological disorders, the cellular and molecular basis of epilepsy is still largely unknown. It is widely assumed that any imbalance between synaptic excitation and inhibition may cause intense neuronal discharges and hyper-synchronous activity in a large number of neurons. Regarding effective drug design, two mechanisms need to be distinguished: *ictogenesis* refers to the mechanisms that trigger an individual seizure, whereas *epileptogenesis* refers to the long-term mechanisms – usually involving a "latent period" of months to years – that transform a healthy brain into a hyperexcitable state with the propensity to develop recurrent spontaneous seizures (i.e., epilepsy). Currently, pharmacotherapy of epilepsy is largely limited to seizure suppression, i.e. to *anti-ictogenesis*. This symptomatic treatment approach has little prospect to affect the underlying causes of the disease. In fact, currently available antiepileptic drugs (AEDs) largely fail to prevent *epileptogenesis* or disease progression. A prerequisite for the prevention of epileptogenesis is a concise understanding of the molecular and histopathological changes that occur between an initial precipitating injury (IPI, i.e., the trigger for epileptogenesis) and the occurrence of the first epileptic seizure. This includes identification of markers for epileptogenesis, identification of subclinical ("silent") seizures that precede the expression of clinical ("convulsive") seizures, and identification of novel molecular targets that might interfere with epileptogenesis.

Despite the fact that most currently available AEDs are effective in suppressing ictogenesis, about a third of all epilepsies – mostly those of focal origin within the temporal lobe (temporal lobe epilepsy, TLE) – remain refractory to pharmacotherapy [1].

Abbreviations: AAT, adenosine augmentation therapy; AAV, adeno-associated virus; AED, antiepileptic drug; ADK, adenosine kinase; AR, adenosine receptor; BHK, baby hamster kidney; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; EEG, electroencephalogram; GABA, gamma amino butyric acid; GAD, glutamic acid decarboxylase; hESC, human embryonic stem cell; hMSC, human mesenchymal stem cell; HSV, herpes simplex virus; IPI, initial precipitating injury; KA, kainic acid; mESC, mouse embryonic stem cell; NMDA, *N*-methyl-D-aspartate; NP, neural precursor; NPY, neuropeptide Y; PES, polyethersulfone; RNAi, inhibitory RNA; SE, status epilepticus; SOD1, superoxide dismutase 1; TLE, temporal lobe epilepsy.

* Corresponding author. Tel.: +1 503 413 1754; fax: +1 503 413 5465.
E-mail address: dboison@downeurobiology.org (D. Boison).

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In addition, many of the existing treatments cause drowsiness, sleepiness, adverse skin reactions, and scores of other side effects [1] that limit their optimal use or dosage. Brain-wide side effects are largely due to systemic drug formulations that unspecifically target neurotransmission, rather than specifically target hypersynchronous bursting. Thus, the requirements for future, more effective therapeutic strategies are: (i) efficacy in pharmacoresistant epilepsy; (ii) prevention of epileptogenesis and targeting of underlying disease processes; and (iii) limitation of side effects by focal mode of action.

2. Failure of neuron-centered pharmacotherapy

Why does current pharmacotherapy fail in over 30% of patients with epilepsy? Most AEDs are based on the *neuron*-centered concept that epilepsy is due to an imbalance between synaptic inhibition and excitation, which in turn is dependent on the equilibrium of gamma amino butyric acid (GABA)-ergic and glutamatergic neurotransmission. Consequently, AEDs directly influence the function of *neurons*: they target GABA-ergic neurotransmission (e.g. vigabatrin, tiagabin, phenobarbital, valproate), glutamatergic receptors (e.g. felbamate, phenobarbital, topiramate), neuronal sodium channels (e.g. phenytoin, carbamazepine, phenobarbital, valproate), neuronal calcium channels (e.g. gabapentin, pregabalin), or affect the presynaptic release of neurotransmitter vesicles (levetiracetam). In summary, all available AEDs directly affect neuron–neuron communication. They largely fail to influence glia–neuron interactions, gap-junction signaling, or inflammatory pathways, processes that are all now known to play an important role in ictogenesis as well as in epileptogenesis.

The majority of AEDs were developed based on conventional screening methods or modification of pre-existing AEDs, rather than on mechanism-directed drug design. Screening methods were largely based on *neuro*-centric epilepsy models and on seizure suppression paradigms. Thus, it is unsurprising that most AEDs are not effective in preventing epileptogenesis. Furthermore, the *neuro*-centric pharmacotherapy for epilepsy of the past decades has failed to provide a major breakthrough in overcoming refractoriness to currently available AEDs. Although some of the newer drugs (e.g. levetiracetam) provide benefit in individual patients with refractory epilepsy, drug refractoriness or intolerability is still a major problem. Several major antiepileptic drugs are substrates for multi-drug transporters [2], thus the failure of current AEDs in a significant portion of patients is aggravated by the development of pharmacoresistance due to increased activity of ABC multi-drug transporters, particularly P-glycoprotein, that become overexpressed during the course of epilepsy and facilitate the efflux and clearance of AEDs [2,3]. Thus, multi-drug transporters in endothelial endfeet of the blood brain barrier constitute an important non-neuronal target for epilepsy therapy. Tariquidar (XR9576) is likely to be a good candidate that appears to inhibit these proteins [3].

In summary, drug development based on the same *neuro*-centric principles as current AEDs is unlikely to provide a major breakthrough in epilepsy therapy. Thus, the search for novel therapeutic concepts and new targets outside classical chemical neurotransmission is currently an area of intensive investigation.

3. Neuron–glial interactions

Information processing in the brain depends on coordinated interplay between cellular circuits comprised of neurons and glia. Neuronal networks communicate via electrically excitable membranes and synaptic contacts embedded in a glial network. Formerly discredited as "glue of the brain," glial cells are now recognized as important contributors to intercellular ion-flux, neurotransmitter exchange, neuromodulation, and metabolite exchange. They function as a long-range communication route that integrates blood vessels and millions of synapses from different neurons into neuronal-glial-vascular units and then into more complex structures connected through a glial syncytium [4]. Thus, glial cells play important roles in coupling neuronal function to the cerebral microvasculature that controls cerebral blood flow (CBF) in the sense that increased neuronal activity requires corresponding increases in CBF. Apart from large stellar processes that stain for intermediate filaments, astrocytes have a multitude of fine processes that have little overlap with processes from other astrocytes and that define individual astrocytic domains, which in rodent hippocampus each contain 300–600 neuronal dendrites and 10^5 synapses [5,6]. Thereby, single astrocytes can sense the activity, and integrate the function, of hundreds of neurons within its domain. In addition, each astrocyte extends at least one process with endfeet surrounding blood vessels of the microvasculature. Therefore, astrocytes are uniquely located to adjust regional CBF to regional energy metabolism. Glia, in particular astrocytes, further serve as key regulators of neuronal function through a mechanism called gliotransmission [7]. Through the release of ATP and glutamate, frequently via regulated synaptic release, a single astrocyte has the unique capability to regulate the activity of hundreds of neuronal dendrites [5,6]. The loss of this astrocytic domain organization appears to play a major role in the pathogenesis of epilepsy [6]. It is therefore not surprising that glial cells are of utmost importance in determining pathological reactions of the brain and that glial function and dysfunction influences the outcome of a wide spectrum of neurological diseases.

4. An astrocytic basis for epilepsy

The central dogma that epilepsy is merely caused by dysfunctional neurons has recently been challenged. An "astrocytic basis of epilepsy" was first proposed by Maiken Nedergaard based on findings that seizures can be triggered by excessive release of astrocytic glutamate that directly targets *N*-methyl-D-aspartate (NMDA) receptors [8]. New studies from several laboratories suggest that non-neuronal (in particular glial) mechanisms comprised of self-reinforcing interplay between dysfunctional energy homeostasis, inflammation, and astrocytic signaling play a critical role in the development of epilepsy [9]. It was recently shown that specific gap-junction subunit proteins allow activity-dependent intercellular trafficking of glucose and its metabolites through astroglial networks [10]. In the absence of extracellular glucose, the delivery of glucose or lactate to astrocytes was able to sustain glutamatergic synaptic transmission and epileptiform activity when astrocytes were connected by gap-junctions [10]. These findings demonstrate that astroglial gap-junctions provide an activity-dependent intercellular pathway for the delivery of energetic metabolites from blood vessels to distal neurons [10]. Thus, excessive neuronal firing in epilepsy may be downstream from pathologically altered neuron–glia interactions.

In addition to mediating gliotransmission, astrocytes can synthesize numerous pro- and anti-inflammatory cytokines during seizures [11]. Astrocytic production of cytokines can lead to pro- or anticonvulsive outcomes, depending on the timing of expression and the receptors activated. Astrocytes also play important roles in modulating inflammatory pathways via purinergic mechanisms. Such findings underline novel functional neuron–glia interactions mediated by cytokines that can contribute to the neuropathology associated with inflammatory reactions in epilepsy [11].